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EVALUATION OF MICROBIAL PROCESSES IN RADIOACTIVE WASTE DISPOSAL

Report No 86-158-K

V.J. Sharpe
Biologist
Biological Research Section
Chemical Research Department

ABSTRACT

The long term safety and integrity of radioactive waste disposal sites proposed for use by Ontario Hydro may be affected by the release of radioactive gases. Microbes mediate the primary pathways of waste degradation and hence an assessment of their potential to produce gaseous end products from the breakdown of low level waste was performed. Due to a number of unknown variables, assumptions were made regarding environmental and waste conditions that controlled microbial activity; however, it was concluded that ^{14}C and ^{3}H would be produced, albeit over a time scale of about 1500 years for ^{14}C in the worst case scenario.

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EXECUTIVE SUMMARY

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Shallow land burial is being considered by Ontario Hydro for the long term disposal of low level radioactive waste and hence the associated technology is undergoing a comprehensive assessment. The long term safety of disposal sites will be affected by radionuclide migration, where formation and subsequent release of radioactive gases may be an important aspect. Microbes mediate the primary pathways of waste degradation and therefore this report contains an evaluation of their potential to produce gaseous products from waste breakdown.

The approach taken was to select the biochemical pathways of importance based on the composition of the waste and then to determine the level of microbial activity by identifying general- and pathway-specific limiting conditions expected to exist within the disposal trench. To encompass a range of possible outcomes, two scenarios were chosen, with complete maintenance of trench integrity as the best case situation, and rupture of the concrete lining with consequent ingress of groundwater as the worst case scenario.

It was concluded that mobilization of ^{14}C and ^{3}H can occur through the processes of cellulose decomposition, methanogenesis, bicarbonate utilization, denitrification, sulphate reduction and degradation of aromatics. Of the general conditions within the trench the severest constraints to microbial metabolism are oxygen-free conditions, a highly alkaline pH caused by the concrete backfill, and the availability of moisture and substrate. Due to a lack of defined information on trench conditions, assumptions had to be made. However, it was concluded that for the best case scenario microbial activity would be reduced but not prevented and the volumes of methane and carbon dioxide produced would be

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considerably less than the maximum theoretical yield. The worst case scenario was similar to, but due to the concrete backfill, not as extreme as that experienced in shallow land burial sites in the United States of America. In these instances, after 11 years of operation, 0.05 to 0.075% of the tritium inventory was being lost annually. A combination of theoretical calculations and field measurements allowed the prediction that within 1500 years all the ^{14}C would have been converted to gaseous forms (CH_4 and CO_2) and released to the atmosphere. Research, involving measurement of microbial cell components and metabolic by-products present in the existing stored waste, is recommended to clarify the extent of microbial involvement in release of radioactive gases.

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TO: Mr. H.N. Isaac
Manager, Nuclear Materials Management
Design and Development Division - Generation

EVALUATION OF MICROBIAL PROCESSES IN RADIOACTIVE WASTE DISPOSAL

1.0 INTRODUCTION

This work, requested by the Nuclear Materials Management Department, forms a part of a comprehensive assessment of the disposal technology for low level radioactive waste generated by Ontario Hydro's nuclear power stations. The long term safety and integrity of disposal sites will be affected by migration of radionuclides, where formation and subsequent permeation of the surrounding geomedia by radioactive gases may be an important aspect. Micro-organisms and the processes they mediate are being considered because they are primarily responsible for the degradation of both organic and inorganic materials, where the resultant metabolic end products are often gaseous.

Supportive evidence for the role of micro-organisms has been provided by the detection of tritiated and carbon-14 contaminated gases vented from shallow land burial sites located in the USA (Kunz, 1982; Francis et al, 1980a). The extent to which microbes are involved in the generation of gases has not been fully categorized for a number of reasons, but it is generally agreed that they do affect radionuclide mobility under these disposal site conditions (Francis et al, 1980b; Champ and Merritt, 1981). However, extrapolation of such conclusions to Ontario Hydro's situation is partially limited by some basic differences in disposal trench design.

The objective of this assessment is to determine the potential for gas production by microbial processes from low level radioactive waste disposal sites to be used by Ontario Hydro. The approach taken was to ascertain the likelihood of microbes being present in the disposal trench. Then the extent of general microbial activity was discussed in relation to the environmental conditions thought to exist within the disposal trench. Based on the composition of the waste, the biochemical pathways and end products of probable importance were identified. To encompass a range of outcomes, two scenarios were considered; one in which total containment was assumed, and a second, worst case scenario,

where rupture of the concrete lining and backfill with consequent ingress of groundwater was assumed. The capacity of microbes to produce acid, and hence neutralize alkaline trench waters, was assessed because of the importance of concrete as a disposal trench design feature. Throughout the report, areas where information was deficient were identified so that recommendations for improving predictions could be made. Suggestions for future research are given. A glossary of terms used in this report is given in the Appendix.

2.0 SOURCES OF VIABLE MICRO-ORGANISMS

Potentially micro-organisms could be transported to a disposal trench from three major sources. They may exist naturally within geological formations and could therefore surround the trench and may be introduced during the construction and trench backfilling phases. The waste itself may contain contaminant organisms from its point of origin. During temporary storage of the compacted cardboard bales damaged plastic casing may allow entry of aerobic microbes attached to particles suspended in air.

Information on the existence of microbes in geological formations has been obtained from examination of rock cores, caverns and mines. Analysis of the former has produced equivocal results because of the potential for introduction of surface organisms during the drilling process. However, European surveys of mines and caves within granitic, clay and oolithic iron ore media have revealed the presence of a wide variety of microbes (West et al, 1985). Aerobic and anaerobic heterotrophic bacterial populations were represented along with anaerobic sulphate-reducing bacteria, which are capable of corroding steel, Thiobacillus species which produce acid under aerobic conditions, and other nutritionally diverse organisms.

To obtain these results selective culture procedures were used which have a disadvantage when nutritionally depleted systems such as deep geological formations are being studied. Such methods tend to isolate only the dominant groups, while oligocarbophilic bacteria adapted for growth in nutrient-poor conditions are lost. More recent studies have determined the presence of bacteria through measurement of cell constituents and hence overcome this problem. In this way bacteria have been obtained from sandy clay (depth 10 m), buff limestone (depth 37.5 m) (Smith et al, 1986) and clay (depth 420 m) (White et al, 1983). From the ratio of extracellular polysaccharide to membrane phospholipid it was determined that these bacteria were viable but under stress. This raises the question of whether microbes are truly active at such depths and hence capable of interacting with buried radioactive waste.

It has been demonstrated that once these stressed microbial communities retrieved from subsurface samples are exposed to more nutrients, then the biomass increased (Smith et al, 1986). An extensive investigation into the microbial content of potential backfill material for waste disposal obtained from Atomic Energy of Canada Ltd (AECL), such as Pembina Mountain clay, Inco tailings, Domtar Seal Bond and various bentonites, revealed a broad spectrum of bacteria representative of those normally found in the soil, which were capable of growth in either aerobic or anaerobic environments (Mayfield and Barker, 1982a). All materials supported significant microbial growth, 10^4 to 10^7 viable cells/g dry weight, when incubated with both synthetic groundwater and a mineral salts solution. It can be seen that micro-organisms are capable of growth within the geomedia in which disposal trenches may be constructed.

Another factor critical to the level of microbial activity is the impact of groundwater. The mineral nutrients required for microbial growth are probably present in the rock matrix, but the organic substrates are thought to be in short supply unless supplied by groundwater. It has been postulated that significant dissolved organic carbon could result from leaching organics out of the upper soil layers (McNabb and Dunlap, 1975). Not all of this carbon may be readily utilized, because an extensive survey of groundwater composition revealed that the bulk of the organics are high molecular weight materials known to be recalcitrant (Barker, 1982).

A lower content of organic carbon in groundwaters of crystalline terrains is to be expected because of the low content of organic molecules in igneous and metamorphic rocks when compared to sediments and sedimentary rocks. However, analyses of groundwater from crystalline rock, sedimentary rock and unconsolidated materials produced average organic carbon values of 5.2 mg/L, 4.8 mg/L and 4.1 mg/L respectively (Mayfield and Barker, 1982b), which indicates that this supposition may be incorrect. Therefore, disposal trenches in crystalline rock may be equally at risk from microbial contamination as trenches placed in sedimentary rocks.

Direct evidence for the existence of viable micro-organisms in groundwaters has been supplied by a number of researchers. Horvath and Elkan (1978) isolated a "variety of bacterial genera" from wells ranging in depth from 320 to 464 metres, and were able to increase numbers a thousand-fold upon addition of organic material. It has been demonstrated that bacteria are able to penetrate intact cores of sedimentary rocks such as sandstone and limestone (Myers and McCready, 1966), and migrate in groundwater through fractured crystalline rock (Allen and Morrison, 1973). Fritz et al (1979) measured high N_2 levels and traces of methane in groundwater percolated systems showing that the microbial

processes of denitrification and methanogenesis had occurred. Furthermore, bacteria have been recovered in water from shallow rock fractures of 20 to 60 metres depth at Chalk River Nuclear Laboratories in Ontario (Champ and Kmet, 1983), and at levels of 10^3 organisms/L from sites in France (West et al, 1985). The result also implies that viable micro-organisms will be transported through the geomedia in groundwater and therefore would be introduced into a disposal trench should breaks in the concrete lining occur.

It is concluded that micro-organisms are distributed throughout both sedimentary and crystalline geomedia to depths in excess of 400 metres. They are also present in groundwater which will aid their migration and enhance growth by supplying nutrients. Subterranean microbial communities, which are similar in composition to surface soil populations, grow slowly, but it has been clearly demonstrated that they are viable and capable of growth. This means that microbes potentially capable of degrading low level radioactive waste will be introduced into disposal trenches during construction and backfilling or through the ingress of groundwater should the trench casing rupture. The other sources of microbes previously cited, contamination of the waste at its point of origin and partial contamination through aerial sources during waste storage, will undoubtedly exist because of the ubiquitous distribution of micro-organisms.

3.0 GENERAL LIMITATIONS OF MICROBIAL ACTIVITY WITHIN A DISPOSAL TRENCH

Once microbes are deposited within a disposal trench their capacity for growth is controlled by a number of key parameters. In particular, certain nutrient and energy requirements must be met. Cellular reproduction needs carbon, nitrogen, phosphorus and sulphur sources plus minerals and an energy source. Biochemical liberation of energy in the absence of light must have: (i) an electron donor, which can be an oxidizable organic compound in the case of chemoheterotrophic organisms or oxidizable inorganics such as molecular hydrogen, ammonia, sulphide and ferrous irons for chemoautotrophic organisms, (ii) an electron acceptor such as molecular oxygen, sulphate, nitrate, carbon dioxide, ferric compounds and simple organics. The carbon source can be supplied by dissolved organic matter, carbonates and dissolved CO_2.

Carbon is the major building block for cells (Stanier et al, 1970), and so its presence is critical for microbial growth. Micro-organisms are varied in their ability to utilize different carbon sources, with heterotrophic bacteria obtaining structural and energy needs from organic carbon, while autotrophic bacteria only use inorganic carbon for growth and have alternative sources for their energy requirements (see Glossary). Heterotrophic activity begins to decrease when dissolved concentrations of

organics drop below 500 to 50 mg carbon/L if it is assumed that organic matter is 50% carbon (Kuznetsov et al, 1963), although some organisms are capable of growth at organic concentrations less than 0.1 mg carbon/L (Zobell and Grant, 1942).

There is a balance between heterotrophic and autotrophic growth, where heterotrophic populations will produce CO_2 which in turn will support autotrophic growth. Alternatively, the production of organic biomass from autotrophic growth can increase the organic carbon content of nutrient-poor environments and hence support heterotrophic growth (West et al, 1985). It can be seen that microbes possess broad metabolic capabilities with diverse nutritional requirements and therefore it is probable that the nutrient status of the waste and repository will be suitable for growth of at least some species (West et al, 1985; Mayfield and Barker, 1982b; Colombo et al, 1982).

This conclusion is strengthened by demonstrations of microbial growth on the contents of leachates removed from shallow land burial sites in the USA (Maxey Flats, Kentucky and West Valley, New York). Populations of aerobic and anaerobic sulphate reducers, denitrifiers and methanogens were successfully isolated (Francis et al, 1980c). Population sizes ranged from 4.0×10^2 - 2.4×10^4 cells/mL for aerobes to 9.0×10^1 - 1.5×10^4 cells/mL for anaerobic bacteria. Additionally, these researchers showed that these microbes could be cultured anaerobically in trench leachates, which indicated that the radionuclides ^{60}Co, ^{85}Sr and $^{134,137}Cs$ with a total activity of 9.6-99.9 Bq/mL and organic chemicals present were not toxic, and that there were sufficient nutrients to allow microbial activity. Further work revealed utilization of carbon and nitrogen compounds under anaerobic conditions where growth did not appear to be limited by the mineral salts concentration (Francis and Colombo, 1978).

The other major constraints on microbial activity are environmental conditions within the trench. There is a dearth of information on microbial activity within disposal trenches and, therefore, much of the data is drawn from studies of microbial survival and growth under extreme conditions.

Temperature is one of the most important environmental factors controlling microbial activity. In general, microbial metabolic activity doubles for each 10°C rise in temperature until thermal denaturation of proteins occurs, which ranges between 60-90°C (Dunlap et al, 1973). However, depending upon the temperature preference of different microbial genera, inactivity, if not cell death, may occur at lower temperatures of 50°C, particularly for psycrophilic organisms. At the other end of the scale are thermophilic bacteria which have been isolated from naturally occurring hot springs. These species will replicate at a temperature of 100°C at atmospheric pressure (Heinen and Lauwers,

1981). Thermophilic sulphate reducers have been observed to produce H_2S within subterranean deposits (2000-4000 metres) at 105°C under approximately 20-40 MPa pressure (Zobell, 1958). More recently, Baross and Deming (1983) isolated thermophiles from 350°C waters emanating from deep sea vents in the Pacific and were able to record growth at 250°C at 26.8 MPa pressure in a laboratory. The minimum temperature limit is determined primarily by a need for water in the aqueous phase and hence growth has been demonstrated at -10°C for two species of Bacillus in a medium containing anti-freeze (Innis, 1975), and down to -18°C for yeast and bacterial species in concentrated fruit juices (Vallentyne, 1963). It can be concluded that some micro-organisms are tolerant of a wide range of extreme temperatures, and that a majority will grow at temperatures between 5°C and 45°C.

In relation to disposal trenches the ambient temperature will be dictated by either the surrounding geomedia or the trench contents. For high level radioactive waste disposal, temperatures may be in the range of 75-100°C (Champ, 1984), but low level radioactive waste is not expected to affect trench temperatures to any great extent. Therefore, temperatures in the subsurface rocks will likely prevail. In the upper 10 m temperatures will roughly correspond to the mean annual air temperature and fluctuate with the seasons. Values of 3°C-25°C have been cited for the United States (Dunlap et al, 1973). Measurement of groundwaters collected from Canadian soils revealed lower temperatures of 10°C-15°C (Champ et al, 1979). It would appear that temperature extremes sufficient to preclude microbial life are not likely to occur in shallow land burial trenches, but that the temperatures may be below optimum and hence depress the rate of growth.

Pressure and temperature are closely related when it comes to assessment of microbial viability. It has been postulated that the maximum temperature for growth increases concomitantly with a rise in pressure and that cell death occurs at pressures in excess of 40 MPa (Marquis and Matsumura, 1978). The isolation of baro tolerant bacteria from high temperature deep sea vents seems to bear this out (Baross and Deming, 1983). Survival of Desulfovibrio desulfuricans has been recorded at the extreme level of 180 MPa (Kuznetsov et al, 1963). Conversely, soil bacteria have been cultivated at low pressures of 10 kPa (Zajic, 1969).

In general, increasing pressure slows the activity of most microorganisms and causes death at approximately 20 MPa (West et al, 1982a). The depth of radioactive waste burial will determine the pressures to which microbes are exposed. Two evaluations of nuclear waste disposal quoted values up to 10-12 MPa for vaults placed at a depth of 1 km, assuming an increase in pressure of 0-1 MPa for every 10 m of depth (Champ, 1984 and Loewen and Flett, 1984). As Ontario Hydro is not currently considering such depths

for low level radioactive waste disposal it is unlikely that microbes will be exposed to pressures within a trench that could limit their activity.

As with other environmental parameters, extreme pH values can be tolerated by certain bacterial species although a majority grow within a narrower range of pH 6.0 to 8.0 (West et al, 1982b). The genus Thiobacillus are well known for their ability to grow in, and generate, acid solutions. This genus has an optimum pH range of 1.0 to 3.0, many growing poorly above pH 7.0 (Vallentyne, 1963). Basophilic microbes, such as nitrogen-fixing soil bacteria, can grow at pH levels greater than 13.0 (West et al, 1982a). Different microbial populations are selected according to pH of the immediate environment where frequently the bulk phase pH is not representative of pH values in microniches where the microbes exist. For example, clays bind cations such as hydrogen ions and hence tend to be more acidic at the solid-liquid interface than further away from the clay particles. Bulk phase pH measurements provide general guidance as to whether the values would limit microbial activity.

Analysis of trench waters from the disposal sites at Maxey Flats, Kentucky covered the extremes of pH 5.5 to 12.8 (Cleveland and Rees, 1981; Clinton et al, 1981; Czyscinski and Weiss, 1981), although the values are generally within pH 6.0 to 8.0 (Colombo et al, 1982). Bales of low level waste from Ontario Hydro's NGS were flushed with 'extract' water with a pH of 7.0. The effluent water collected was mildly acidic with a pH between 4.0 to 5.0 (A.S. Williamson, personal communication). Such pH values are not expected to prevent microbial metabolism despite being somewhat lower than the general optimum of a near neutral pH level. Therefore, there is some definition of the conditions within the bales of compacted waste. However, external to the waste packages, and maybe within them at a later stage should moisture migrate from the backfill, will be a highly alkaline environment created by the surrounding concrete. It is this design feature which sets the Ontario Hydro disposal trenches apart from almost all other design concepts, and which may be the primary controlling environmental characteristic as regards microbial activity. The integrity of the bales plays a crucial role. Should moisture from the highly alkaline (approximately pH 12.0) backfill reach the inside of the waste then, despite some neutralization by the slightly acidic waste moisture, the pH will rise. If the pH within microniches of microbial growth becomes more alkaline than pH 8.0 then there will be considerable reduction in microbial activity. The slower the progressive change in pH the greater will be the capacity of a microbial population to adapt to the altered conditions and continue breaking down the waste materials. However, as the waste system is enclosed, the capacity of the population to adapt will be limited by the lack of access of other soil micro-organisms.

There is one disposal trench, for which data is available, that is similar in composition to the Ontario Hydro radioactive waste trenches. At Maxey Flats, Kentucky, trench 33L4 is atypical in that cement was used as a solidification agent. Trench leachate collected from this trench had a pH of 12.1 (Pietrzak et al, 1982). If this pH level was to prevail throughout the waste bales in the Ontario Hydro trenches then microbial activity would be severely curtailed.

The redox, or oxidation reduction, potential of a system, that is the capacity to accept or to donate electrons, is obviously closely linked to the pH of the environment. The redox potential (E_h) measured in millivolts, both dictates the type of microbial metabolism and is itself altered as a result of microbial activity. Conditions in which microbes can grow have been cited by Vallentyne (1963) as ranging from an upper E_h limit of +850 mV at pH 3 for iron bacteria to a lower limit of -450 mV at pH 9.5 for sulphate-reducing bacteria. But each species of microbe usually grows within a relatively narrow range of redox values and hence the control of metabolic pathways. Microbial oxygen consumption progresses until O_2 has been depleted at an E_h of +350 to +300 mV. Then other oxidized chemical species will be used in the sequence NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2 and N_2. Nitrate reduction occurs at an E_h below +200 to +100 mV (Bailey and Beauchamp, 1971). Manganese and iron follow nitrate as electron acceptors, with ferric reduction occurring below +240 mV. Sulphate reduction occurs after iron reduction at an E_h around -100 mV (Postgate, 1979). Below -150 mV, reduction of CO_2 to methane occurs (Loewen and Flett, 1984).

The E_h conditions prevailing within disposal trenches will vary depending upon the age after trench closure. Once the trenches have been sealed, a combination of remaining aerobic microbial growth and possible corrosion of iron canisters will render the atmosphere anoxic (oxygen free). This is the second key parameter which will determine the type and extent of microbial activity. This scenario was predicted for low level waste disposal trenches in the USA (Colombo et al, 1982) and was confirmed when redox potentials of leachates collected from these trenches were found to lie between 0 and -100 mV (Dayal et al, 1984). These values relative to the normal conditions for groundwaters in the area (+280 mV) indicate that chemically reducing conditions exist within the trenches.

Water is obviously a prerequisite for life, but microbes can exist even when its availability is greatly restricted such as in oil emulsions in subterranean deposits and jet fuels (Ehrlich, 1972; Zajic, 1969), or in areas of high salinity (up to 50% salt by weight) (West et al, 1982b). Some bacteria and fungi survive periods of drought by producing spores. In this state of dormancy they can survive for decades but, alternatively, will

not be decomposing waste material either. The lowest relative humidity at which microbial growth is possible depends upon the species, but most soil contains only hygroscopic water (McLaren and Peterson, 1967). Currently this information is not directly translatable to the Ontario Hydro situation as relative humidity values for the compactable low level radioactive waste have not been measured. Observation of 1072 bags of waste revealed that about 11% contained visible moisture (Ontario Hydro Research Division Report, 1985b). This means that within a bale consisting of 50 to 70 bags approximately 7 bags will be 'wet.' The 'dry' bags may contain moisture, but if condensation is not visible the amount of water may be at the hygroscopic level, and hence microbial decomposition within these bags will be significantly restricted.

Another important parameter that can effect microbial activity is ionizing radiation. Micro-organisms tend to be more resistant than higher organisms, but again the degree of susceptibility varies with species. The effect of ionizing radiation also depends on conditions at the time of exposure. For instance, anaerobic conditions, which are likely present in Ontario Hydro's low level radioactive waste (LLW) disposal trenches, tend to reduce the sensitivity of bacterial cells (Mayfield and Barker, 1982b). The stage of microbial life cycle, level of nutrition, dose, exposure time and post-irradiation conditions are other factors to be considered (Nassim and James, 1978). Extreme tolerance has been exhibited by such organisms as Micrococcus radiodurans which can withstand doses of gamma irradiation as high as 5 kGy (Nassim and James, 1978), or the red bacterium isolated by Engvild and Berenstein (1975) that survived gamma doses between 10 and 35 kGy. Most microbes exhibit 90% cell death when exposed to 3 to 4 kGy (Mayfield and Barker, 1982b).

Studies of microbial resistance to radio-isotopes in trench leachates from shallow land disposal sites at Maxey Flats would seem to indicate that microbial populations are more sensitive than the values cited above indicate. Bacteria isolated from trenches were grown in media containing ^{60}Co, ^{85}Sr and $^{134,137}Cs$ at total activity concentrations of about 10 Bq/cm^3, which were similar to those measured within the trenches by Weiss and co-workers (Weiss et al, 1979). Although no inhibition of growth was observed at this level, growth was restricted at 10 kBq/cm^3 (Francis et al, 1980c). However, other radio-isotopes were less damaging as these same researchers successfully cultured methane bacteria on a synthetic medium containing 2.4 MBq tritium/cm^3 (Francis et al, 1980b). These values are useful for comparison with the situation at Ontario Hydro. Many of the bags examined generally had a low specific activity. The beta and gamma radionuclide activity was generally attributable to isotopes with a half-life of less than one year so after 10 years no bale would exceed 56 MBq. The tritium activity of compacted waste was

estimated to be between 311 to 418 GBq/m^3 whereas ^{14}C activity was thought to range between 30 to 200 kBq/m^3 (Ontario Hydro Research Division Report, 1985b). Depending upon the age of the waste at burial, it is possible that microbial waste decomposition would be curtailed in some bales due to the level of radioactivity. However, it is concluded that radionuclides would not prevent microbial growth in many of the waste bags.

In summary, it appears that microbial populations are capable of growth over a wide range of environmental conditions, the extremes of which are unlikely to be present in Ontario Hydro's disposal trenches. Hence, such factors as moisture and radioactivity will reduce the rate and extent of growth/waste breakdown, but not prevent it entirely. Once microbial populations are established, the two major parameters which will dictate the type of microbial activity are absence of oxygen and alkaline pH. Although, the latter may not exert selective pressure consistently throughout a trench depending upon the distribution of waste, moisture levels and trench integrity. The interrelationship between environmental conditions and specific biochemical pathways are discussed in the following sections.

4.0 MICROBIAL PATHWAYS FOR GAS PRODUCTION

The type and concentration of nutrients in the trench, that is, the waste composition, will dictate the biochemical pathways that are feasible. Analysis of the plastic-wrapped cardboard bales revealed an extremely heterogeneous composition. The physical composition was classified into three main types. Plastic comprised between 15% to 62% of the waste, paper ranged between 16% to 45% and other varied from 22% to 52% (Ontario Hydro Research Division Report, 1985b). The miscellaneous component covered sweeping compound, vermiculite, fibreglass insulation, metal pieces, electrical cable, rubber boots, animal carcasses, food, gloves, cans, glass and plastic bottles containing oils and aqueous fluids. Suffice it to say that these materials provided some major nutrients for microbes in the categories of proteins, lipids, polysaccharides and aromatic compounds.

4.1 Cellulose Decomposition

As a major carbonaceous component the degradation of cellulose is a key step in the breakdown of the waste, particularly as radionuclide mobilizing processes such as methanogenesis are dependent upon the liberation of CO_2 or H_2 and fatty acids from prior metabolic pathways. There is a wide range of fungi and bacteria which can degrade cellulose under aerobic conditions, but the organisms capable of anaerobic breakdown are far fewer in number. Although commonly found in soils, the limited number of anaerobic bacterial genera of interest, *Actinomyces* and *Clostridium*, will decrease the potential for rapid degradation. Additionally,

because organic carbon sources are required as carbon sources and electron acceptors in fermentation pathways, the process is less energetically favourable than aerobic growth. This means that for the same size of population anaerobic bacteria require more substrate than aerobic bacteria and so the efficiency of cellulose breakdown will be reduced. Available surface area of the substrate also limits degradation and so the cardboard boxes in particular provide an unfavourable surface for attack, unless the constituent fibres swell due to moisture.

Another crucial factor controlling microbial metabolism is the energy:nutrient ratio, which for convenience sake can be expressed as carbon:nutrient ratio. For biosynthetic processes to occur, the uptake of nutrients can only occur through expenditure of energy derived from degradative pathways. Decomposer organisms possess relatively narrower carbon:nutrient ratios than the substrate they wish to utilize and hence nutrients in general, and nitrogen in particular, frequently limit growth yields. To define one upper limiting ratio is open to criticism for a group of organisms as diverse as microbes, but as a guide, wood, which is only very slowly degraded, has a C:N ratio of 157 (Swift et al, 1979). As mineralization progresses, releasing CO_2 to the atmosphere, and nutrients such as nitrogen are immobilized, the ratio will improve to a point (C:N $\lesssim$ 25) where nitrogen is no longer limiting. In the case of cellulosic waste, the implication is that once degradation has been initiated the process will continue, albeit slowly.

All of these parameters can increase the lag time before initiation of degradation and decrease the rate of breakdown. However, as there is no definitive information on nutrient and environmental factors, it is not possible to provide a quantitative assessment of gas production.

In terms of radionuclide mobility, cellulose degradation will not have a direct effect, but as a result of this activity more accessible intermediate substrates and CO_2 will be produced which will provide energy and nutrient sources for other microbial groups, specifically the methanogenic bacteria.

4.2 Methanogenesis

Methane formation will only occur if organic breakdown produces suitable nutrients and energy sources (fatty acids, CO_2, H_2) (Ontario Hydro Research Division Report, 1981) and the oxidation-reduction potential (E_h) is sufficiently reducing (-200 to -1000 mV) (Colombo et al, 1982). Production of methane and carbon dioxide, the end product of methanogenesis, increases with moisture content, with an optimum around 60-80 per cent (Van Engers, 1982). Higher temperatures also enhance activity with methanogenesis occurring between 4-45^{o}C with an optimum of 35-42^{o} (Weiss

et al, 1982). Should highly alkaline waters reach the degraded carbonaceous waste then inhibition of methane formation could occur as activity has only been measured over a pH range of 5.4-8.4 (Rees, 1980), with the exception of Methanococcus vanniellii which possesses an optimum between pH 7.4-9.2 (Stafford et al, 1980). It would seem that the environmental conditions probably present within the disposal trench would severely reduce the amount and rate of methane generated but not its existence, assuming that prior degradative processes have occurred.

The potential for incorporation of radionuclides into methane and carbon dioxide is high because the fatty acid precursors may originate from contaminated organic wastes and the CO_2 and H_2 are likely to contain ^{14}C and ^{3}H isotopes. There is a great deal of evidence that, as a result of microbial activity, radioactive methane and carbon dioxide are vented from shallow land burial sites in the United States (Kunz, 1982; Lu and Matuszek, 1978). However, a lack of precise information on original trench inventory has precluded quantitative assessments of the extent of radionuclide 'carryover.'

Quantitative evaluation of the degree and rate of conversion of waste to gaseous end products is hampered by similar limitations. Theoretically the yield of methane (CH_4) and carbon dioxide (CO_2) from glucose, which is the basic unit from which cellulose is constructed, is given by:

1 kg $C_6H_{12}O_6$-->0.25 kg CH_4+0.69 kg CO_2+0.056 kg dry biomass+632 kJ heat

(after Pirt, 1978). The CH_4 and CO_2 component is equivalent to 0.7 m^3 gas at standard temperature and pressure. Comparison between theoretical yields (0.7 m^3 for CH_4 and CO_2 kg^{-1} glucose), optimized anaerobic digesters (0.23-0.32 m^3 gas kg^{-1} wet refuse) and experimental soil columns more closely simulating landfill conditions (0.0005-0.039 m^3 kg^{-1} refuse [Rees, 1980]), illustrates the decrease in conversion efficiency under 'real life' situations. It should also be noted that the ratio of CH_4:CO_2 produced varies greatly depending upon the nature of the original carbon source and temperature (Ontario Hydro Research Division Report, 1981).

The design of the shallow land burial sites in the USA has resulted in ready ingress of water, so that despite a lack of readily degradable waste, the conditions supported a relatively high level of degradative/gas forming capacity. Trench gas samples have been found to contain primarily CH_3T and HTO, but also HT, other tritiated hydrocarbons, $^{14}CO_2$, $^{14}CH_4$ and other ^{14}C-hydrocarbons (Lu and Matuszek, 1978).

The various methods used to calculate and present data help to confound attempts at predicting radioactive gas releases by

comparing theoretical and field-derived data. In the case of disposal trenches in the USA (West Valley, New York), measurements of concentrations of CH_3T (29.6 Bq cm^{-3}) and trench gas pressure were developed into a model to produce estimates of the extent of release of tritiated methane annually (148 Bq) (Lu and Matuszek, 1979). For the same trench (5), another estimate using an incidental gas production rate combined with the assumption that the effluent gas consisted predominantly of methane, which is unlikely as the ratio of methane to CO_2 decreases as the organic carbon content of the waste drops, gave an annual total release of 222 GBq CH_3T (Lu and Matuszek, 1979). Values for the original inventory are required to ascertain the fraction of trench tritium inventory released as gas. These researchers produced a figure of 296 TBq for trench 5 based on burial and pumping records and an assumption that miscellaneous waste contained no tritium. The latter assumption may result in an underestimate of tritium inventory up to 50%. However, using the values given it can be calculated that 0.05-0.075% of the tritium inventory is released annually from a trench approximately 11 years after disposal of the waste. If one assumes a consistent rate of loss then the entire tritium inventory will be transmitted to the atmosphere within 1300-2000 years. The implication for tritium is not so critical with a half life of about 12 years, but is more serious for ^{14}C (half life of 5568 years). In another assessment of the same disposal site it was estimated that it would take 1500 years for all the carbon to be converted to CH_4 and CO_2 (Kunz, 1982).

For low level waste sites in the USA, it can be seen that irrespective of the accuracy of assumptions and models used to calculate the rate and extent of radioactive gas releases, loss of radioactive gases has been recorded. The proposed design of the Ontario Hydro disposal trenches, in particular the use of concrete backfill, should increase the time before initiation of microbial degradation and hence methanogenesis and retard the rate of gas production. However, in the light of the level of gas lost from trenches in the USA it is concluded that radioactive methane and carbon dioxide will be released from Ontario Hydro sites in the worst case scenario. This scenario presumes the ingress of groundwater, which would carry nutrients, and is similar to conditions experienced in the USA. The limiting factor in this case would be the severe alkalinity of the water, although the impact can only be estimated. If the entire waste was at pH 12 then methanogenesis would not occur as bacteria do not maintain a large difference between internal cytoplasmic pH and external pH (Swift et al, 1979). However, it is well known that micro-organisms are often active within microsites where the pH is considerably different to the bulk phase pH (Swift et al, 1979).

In fact, this situation is indicated by pH data recorded for water samples collected from shallow land burial sites in the USA, where general values for all the sites ranged from pH 2.2-12.4 (Weiss and Colombo, 1978) and more specifically for trenches at Maxey Flats, Kentucky, pH 5.5-12.8 (Clinton et al, 1981) and pH 2.2-12.2 (Cleveland and Rees, 1981). Despite pH extremes recorded for bulk water samples, it was apparent that microbial activity and radioactive gas releases existed. The variability in pH conditions for essentially similar trenches at the same burial site is also of note.

4.3 Bicarbonate Utilization

As bicarbonate accounts for a large proportion of the inorganic carbon and ^{14}C inventory, the processes associated with its mobilization are important. This form of carbon is unusable by heterotrophic organisms and can only be metabolized by autotrophs, which contain their structural carbon from inorganic sources (CO_2, HCO_3^-, CO_3^{2-}) and the necessary energy by oxidizing reduced inorganic compounds (H_2, H_2S, NH_4^+, NO_2^-, Fe^2, Mn^{2+}) (Alexander, 1971). The source of oxidizing power for autotrophs is usually oxygen and so under anoxic trench conditions the number of microbes capable of utilizing an oxidizing agent other than O_2, termed anaerobic respiration, is small.

One pathway is the reduction of CO_2 using H_2 to produce CH_4, both precursors potentially providing for conversion of radioactive species to gaseous forms. This pathway is combined with assimilation of inorganic carbon into the cell that may temporarily immobilize ^{14}C, but has converted it to an organic form, which, when the cell dies, will be released and then available for utilization by a greater range of microbes. A methanogenic bacterium, Methanobacterium thermoautotrophicum, is capable of this type of metabolism, although it will not initiate degradation as the bacterium requires highly reduced conditions. Another organism capable of mobilizing radioactive bicarbonate is an autotroph, Thiobacillus denitrificans, which uses nitrate as an oxidant instead of oxygen. At NO_3^- concentrations found in groundwaters in the Canadian Shield (approximately 0.4 mg NO_3^- L^{-1}) it was thought that the activity of this bacterium would be restricted (Loewen and Flett, 1984). This would not be the case for some of the USA trenches, our worst case scenario, where nitrate values ranged from <0.05-21 mg NO_3^- L^{-1} (Clinton et al, 1981). These organisms will also increase the pool of organic carbon, thereby enriching the nutrient environment which will in turn encourage growth of a broader spectrum of organisms. Similarly, other organisms capable of using inorganic carbon under anaerobic conditions, such as the nitrogen fixing Xanthobacter autotrophicus, and nitrate-reducing Paracoccus denitrificans (Ontario Hydro Research Division Report, 1985a) will initiate the bicarbonate mobilization process.

4.4 Degradation of Organic Compounds

A major source of nutrients for microbial growth in disposal trenches is organic compounds, which are generally the most amenable to breakdown by the largest group of micro-organisms using fermentation and anaerobic respiration processes. Cellulose degradation and methane generation, which fall into this category, have been dealt with elsewhere. There are multiple pathways by which organics can be degraded anaerobically; they include denitrification, followed by sulphate reduction and then methane generation. A primary indicator of the potential for microbial activity is the concentration of dissolved organic carbon (DOC) available for conversion. Analysis of trench waters retrieved from the Maxey Flats facility in the USA showed values for DOC ranging from 5.1-1900 mg L^{-1} (about 0.005-2.0 w/v) (Clinton et al, 1981). General heterotrophic activity is thought to decline at DOC concentrations between 50-500 mg carbon L^{-1} (Loewen and Flett, 1984), although for some marine organisms growth has been demonstrated at 0.1 mg carbon L^{-1} (Zobell and Grant, 1942). A comparison of DOC values seems to indicate that, in general, microbial activity would not be limited under conditions equated with the worst case scenario. In an assessment of the capacity of backfill material (clays and shales) to support microbial growth which contained 0.5-5 per cent organic carbon w/w (assuming that organic matter is 50% carbon - Kuznetsov et al, 1963), a similar conclusion was reached (Mayfield and Barker, 1982a). To determine whether microbial growth would be restricted under conditions expected for the best case scenario, more specific information is needed.

Denitrification, an anaerobic process whereby nitrate acts as an oxidizing agent in the breakdown of organics, is obviously limited by the availability of nitrate. It is common that nitrogen will restrict microbial activity as many environmental systems are nitrogen limited, and in one study it was felt that denitrification would not be an important process in many disposal trenches at the Maxey Flats site (Dayal et al, 1984).

Of more importance is sulphate dissimilation where sulphate is reduced to sulphide and organic compounds are oxidized, often only partially to produce a pool of fatty acids and CO_2. The accumulation of these products is significant as they may serve as substrates for methane formation. Additionally, these organisms may temporarily reduce tritiated gas levels because they can incorporate hydrogen while using it as an energy source. Sulphate-reducing bacteria are ubiquitous within terrestrial and aqueous environments and so it is difficult to pinpoint a sulphate concentration that would limit their growth. However, even in an enriched culture medium designed to grow these bacteria the sulphate concentration used ranges from 50-250 mg L^{-1} (Postgate, 1979). Measurement of sulphate levels in trench leachates at

Maxey Flats, USA, may be somewhat misleading as low values could indicate that sulphate reduction had already occurred leaving minimal sulphate behind. However, sulphate concentrations in the trenches ranged from <1.0-1700 mg L^{-1} indicating that some sites would support extensive sulphate reducer populations as long as E_h is poised at -100 mV or less (Postgate, 1979). Data for this critical parameter show that chemically-reducing conditions are present in all monitored trenches at Maxey Flats (0 to -100 mV) but that only one trench was below -100 mV (Dayal et al, 1984). Therefore, parallels between these trenches and the worst case scenario indicate that at least some sulphate-reducing activity, and hence a major degradative pathway which also acts as a precursor for methane generation, would occur. A similar conclusion was reached in a microbiological study of trench leachates where it was demonstrated that sulphate-reducing, denitrifying and methanogenic bacteria were not only present but all capable of growth on the leachates (Dayal et al, 1984). Further supportive evidence is supplied by a laboratory study of microbial activity within trench leachates where anaerobic degradation of organic compounds occurred, albeit slowly (Francis, 1981).

The remaining pathway of interest is the potential for breakdown of aromatic compounds, which are normally degraded under aerobic conditions. It is well recognized, and has been demonstrated, that aromatics become more resistant to microbial attack upon creation of anaerobic conditions (Tate, 1979). It has been frequently cited that anaerobic micro-organisms are capable of aromatic ring decomposition (Guyer and Hegeman, 1969; Dutton and Evans, 1969), although it is not mentioned that the organisms studied require light and therefore could not function under trench conditions. More importantly, there is evidence that a wide range of organisms, many not fully characterized, will degrade aromatics anaerobically during nitrate respiration (Williams and Evans, 1975) and methane formation (Healey and Young, 1979; Balba and Evans, 1977; Mah et al, 1977). It would appear that in these cases consortia of microbes are necessary for complete degradation of aromatic nuclei, making it even harder to pinpoint limiting conditions. It has been concluded by others (Colombo et al, 1982) that under trench conditions anaerobic breakdown of aromatic polymers would be minimal.

4.5 Breakdown of Plastics

Plastics are highly recalcitrant materials and there is little information on their breakdown, particularly under anaerobic conditions. Leaching, which presumes high moisture levels in the disposal trenches, weakens plastics by removing plasticisers thereby increasing exposure of the polymer backbone to enzymatic and chemical action (Rees, 1980). It is to be expected then, that there will be little microbial decomposition of plastic. One important observation in sanitary landfill sites was that

plastics remained impermeable to water after a year's exposure to landfill leachate (Rees, 1980). In terms of low level radioactive waste disposal, it seems possible that, within plastic casing, microbial activity would be unhindered by the highly alkaline pH environment of the concrete backfill for some time after trench closing.

5.0 ACID PRODUCTION

Assuming that there will be sufficient moisture for migration of water from the concrete backfill to the buried waste, the highly alkaline pH would likely inhibit much of the potential microbial activity. A second assumption is that the organisms would actually come into contact with this water, which would probably only occur under the worst case scenario, in which case this may be the major limiting factor for this scenario. Under the best case situation excess water is not anticipated, which may actually be detrimental from a safety standpoint as large volumes of alkaline water may not reach the microbes. The basis for this conclusion is that microbes can produce acid on a local basis so that within microsites there is potential for neutralization of alkalinity. Partially degraded carbonaceous compounds include acids and alcohols (Colombo et al, 1982) and acids derived from protein metabolism (Rees, 1980). More specifically, the Thiobacillus species associated with nitrate reduction will increase acidity (Loewen and Flett, 1984; West et al, 1985) and acetogenic bacteria produce acetic acid from carbon dioxide and hydrogen under strictly anaerobic conditions (Ontario Hydro Research Division Report, 1985a). However, there are microbial processes that produce alkalinity, such as sulphate reduction, de-amination of amino acids and denitrification, to counteract the acidity. Overall, microbial acid production cannot neutralize all the backfill alkalinity, but this would not be necessary to allow some organisms to be metabolically active within microsites.

6.0 CONCLUSIONS

The ability to derive conclusions from the available information is limited by two main factors: a lack of specific data on environmental conditions and the existence of too many undefined variables. The environmental conditions within Ontario Hydro's disposal trenches are not defined, neither are the fluctuations in biological and chemical parameters likely to occur during trench operation. Even improved description of such conditions will not allow determination of the accessibility of the wastes to microbes, a critical factor (West et al, 1985) which will likely vary considerably within individual waste packages. There is very little information on non-optimized growth rates of micro-organisms within any environment (Mayfield and Barker, 1982b), let alone on the activity of microbial consortia performing complex interacting degradative pathways within disposal

trenches. Therefore, estimation of the extent and rate of release of radioactive gases to the environment from an undisturbed Ontario Hydro low level waste site, the best case scenario, is highly speculative at best. However, it could be said that:

i) initially viable micro-organisms will be present in some of the waste packages, and that some of these organisms can function under oxygen-free conditions;

ii) judging from the visual observation of moisture in about 11% of the waste bales, complete exclusion of water, and hence of microbial growth, is not likely;

iii) that mobilization of ^{14}C and ^{3}H can potentially occur via the processes of cellulose decomposition, methanogenesis, bicarbonate utilization, denitrification, sulphate reduction and degradation of aromatics;

iv) that the extent of microbial activity cannot be determined from the current available literature;

v) that theoretical yields of methane and carbon dioxide from glucose, ie from cardboard which represents the bulk of the waste, is 0.7 m^3 gas per kg glucose, but that this value is greatly reduced under sanitary landfill conditions, which are considerably more conducive to microbial activity than radioactive waste disposal sites.

In contrast, the worst case scenario, as represented by the shallow land burial sites in the USA, allows for more certain predictions. In assessments of microbial activity, both controlled experiments using leachate removed from these trenches (Francis, 1981) and field-derived data from analysis of leachate components (Dayal et al, 1984) produced the conclusion that microbes were both present and active within the disposal sites. As a result of their metabolism radioactive gases were being released to the atmosphere. For shallow land burial sites in use within the USA, a combination of theoretical assessments and field measurements revealed that:

i) trench gas samples were found to contain mainly CH_3T and HTO, but that other tritiated forms plus $^{14}CO_2$, $^{14}CH_4$ and ^{14}C-hydrocarbons were present;

ii) estimates for release of CH_3T ranged from 148-222 GBq CH_3T from a single trench per annum;

iii) 0.05-0.075% of the tritium inventory is released annually from an 11-year-old trench;

iv) it was thought that within 1500 years all the carbon would have been converted to methane and carbon dioxide with the consequent release of ^{14}C.

However, it should be remembered that one of the major differences between the design of these trenches and those to be used by Ontario Hydro is the presence of concrete backfill in the latter. This one factor would appear to increase disposal trench safety/stability from a microbial standpoint. As a result of waste compartmentalization within the backfill, microbial gas production will be retarded because limitations to microbial activity are less likely to be overcome if there is no bulk mixing of waste components. Alternatively, the composition of the waste is heterogeneous and the environmental conditions within the disposal trench not overly rigorous. Therefore, in the absence of any definitive evidence to the contrary, it is concluded that microbes will degrade the waste and produce radioactive gases over a long period of time. Use of conventional measures to kill bacteria are impractical. Complete mixing of waste and biocide would be required if chemicals are to be effective and handling of compacted waste necessary for autoclaving/sterilizing would be extremely difficult. Gamma irradiation is used as a treatment for contaminated materials, but not on the scale or form presented by compacted low level radioactive waste. Therefore, a control measure, which falls within the current disposal strategy, which would prevent the eventual slow release of radioactive gases as a result of microbial activity, is not known at present.

7.0 RECOMMENDED RESEARCH

An evaluation of the literature has revealed a lack of definitive evidence on microbial activity within the type of trench to be used by Ontario Hydro. Hence, all the conclusions are to some extent open to interpretation. Two key questions require clarification:

1) Does microbial degradation of low level radioactive waste occur unequivocally under trench conditions presented by the Ontario Hydro design?

2) What is the extent and rate of gas production by microbes?

A primary consideration in the development of research projects is to provide a link between realism, the extent of experimental control, and hence precise quantification of results and the duration of the study. In both of the above cases a 'black box' approach could be taken, whereby simulated waste repository conditions would be devised and samples of real compacted waste kept under sterile or non-sterile conditions. Gaseous products would be monitored to see whether the presence of a natural population

of contaminant organisms had any effect, and to what extent. A problem arises with the extent of reality of the simulated trench conditions. The more accurate the representation, the longer the delay will be prior to initiation of degradation. This period may be prolonged in experimental terms, but still inconsequential in terms of assessing trench safety where ^{14}C release should be contained for thousands of years. Whether enhancement of test conditions to speed up the work, and hence extrapolation of assumptions, is reasonable is a moot point.

Alternatively, pure kinetic studies of the ability of single species to degrade specific wastes under various conditions to determine the limiting environmental factors and limiting concentrations of critical nutrients is not recommended as this is too dissimilar to real life, mixed microbial population, sub-optimal conditions. Additionally, the critical question of substrate availability/microbe-waste interaction is not being addressed at all in this sort of approach. While it is being considered only partially in the less rigidly controlled, but more realistic study outlined in the previous paragraph.

Another approach which may prove more fruitful is to examine the existing waste which has been temporarily stored above ground in warehouses for evidence of microbial degradation. Selection of both microbial cell constituents (eg muramic acid - a cell wall component), and metabolic by-products (eg fatty acids), possibly using gas chromatography-mass spectrometry, would indicate whether microbes have been capable of breaking down the waste under the far less restrictive storage conditions. If this proves to be the case, then it will be known: (i) that microbes have started to degrade the waste, (ii) that they will continue once placed in a disposal trench until the oxygen is depleted, at least, (iii) the chances of further breakdown are highly likely, (iv) the buried waste radioactive inventory may be far less than anticipated due to pre-disposal microbial action. Such measurements made from waste bales stored for different periods of time, there are some as old as 20 years, will provide information on the rate of microbial breakdown of waste. Field monitoring of radioactive gaseous releases would give useful supplemental data. Other questions which should be considered are: (i) the effect of waste degradation and hence compaction within the backfill upon the structural integrity of the trench, (ii) the rate of gas transport through various thicknesses of concrete. However, the

microbial aspects should be clarified before this work is embarked upon.

Approved by:

O.A. Kupcis
Manager
Chemical Research Department

Prepared by:

V.J. Sharpe
Biologist
Biological Research Section

Reviewed by:

F.S. Spencer
Acting Supervising Biologist
Biological Research Section

VJS:rv

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APPENDIX 1

GLOSSARY

Aerobic	(of an environment) where there is free oxygen at partial pressures similar to those found under normal atmospheric conditions
Anaerobic/Anoxic	(of an environment) where there is an absence of free oxygen
Autotroph	An organism that uses carbon dioxide as the sole source of carbon (for growth), while energy for growth may be supplied by light (photoautotroph) or derived for inorganic substances (chemoautotroph)
Baro Tolerant	Of microbes which can reproduce at greater than atmospheric pressures
Chemoautotroph	An organism that obtains its energy and carbon from chemical reactions involving strictly inorganic sources, excluding light
Chemoheterotroph	An organism that obtains its carbon from an organic carbon source while its energy may be derived from chemical reactions involving either inorganic or organic sources, excluding light
Cultures	A growth of a particular type of micro-organism on or within a solid or liquid nutrient medium
Cytoplasmic	Essential living matter of any cell inside a cell wall but outside the nucleus
Electron Acceptor	Oxidizing reagent - a compound or element which gains an electron and/or hydrogen atom
Electron Donor	Reducing agent - a compound or element which loses an electron and/or hydrogen atom
Facultative	(adj-optional) Refers to the ability of an organism to adapt to an alternative life style or mode of nutrition
Heterotroph	Organism which requires a source of exogenous organic compounds for growth and reproduction

Hygroscopic	(of water) which is bound very strongly to soil particles during drought and is generally unavailable to plants and microbes
Methanogenic	(adj of bacteria) which are capable of energy production through the process of anaerobic oxidation of hydrogen gas, using CO_2 as the electron acceptor, the metabolic products of which are methane (CH_4) and water
Methylotroph	(adj of bacteria) which use methane as the sole source of carbon through a strictly aerobic pathway which has not been fully elucidated
Micro-Aerophilic	Refers to oxygen concentrations which are much less than normal atmospheric levels but are not anaerobic
Obligate	Refers to any state or condition which is an essential attribute of a given organism
Oligocarbophilic	Of a microbe - which is only capable of growth under conditions of low nutrient concentration
Psychrophile	An organism capable of growing at $0^{o}C$ and with an optimum growth temperature below $20^{o}C$
Thermophilic	Pertaining to optimum temperatures for growth of micro-organisms which exceed $45^{o}C$
Vegetative	Of cells - which are involved in nutrition and growth, ie not reproductive or dormant forms